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PERMANENT GENETIC RESOURCES

Isolation and characterization of microsatellite loci from the European corn borer, *Ostrinia nubilalis* (Hübner) (Insecta: Lepidoptera: Crambidae)

KYUNG SEOK KIM, BRAD S. COATES, RICHARD L. HELLMICH, DOUGLAS V. SUMERFORD and THOMAS W. SAPPINGTON*
USDA-ARS, Corn Insects and Crop Genetics Research Unit, Genetics Laboratory, Iowa State University, Ames, IA 50011, USA

Abstract

Few useful microsatellites are available for population studies of the European corn borer, *Ostrinia nubilalis* (Hübner). An enrichment strategy was used to develop microsatellite markers for *O. nubilalis*, and over 500 positive clones were isolated. Seventy-five contained unique microsatellites, 10 of which were polymorphic with discernable polymerase chain reaction products. The 10 loci were surveyed for variability in 72 wild individuals from central Iowa. Five loci showed no deviation from Hardy–Weinberg proportions, and all were successfully cross-amplified in the related Asian corn borer, *Ostrinia furnacalis*. These loci represent a significant addition to microsatellites appropriate for population studies of *O. nubilalis*.

Keywords: European corn borer, microsatellites, molecular markers, *Ostrinia furnacalis*, *Ostrinia nubilalis*, population genetics

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The European corn borer, *Ostrinia nubilalis* (Hübner), is a major insect pest of corn in North America and Europe, and is a model species for studying questions of evolution, hybridization, and sympatric speciation (Dopman *et al*. 2005; Malausa *et al*. 2005). There is widespread interest in characterizing genetic structuring and gene flow in this lepidopteran species. Developing microsatellites that are useful for population studies is particularly difficult for Lepidoptera (Meglecz *et al*. 2004; Zhang 2004). There are a few reported microsatellites for *O. nubilalis* (Coates *et al*. 2005; Dalecky *et al*. 2006), but the presence of null alleles and instability of flanking regions limits the utility of many of these loci for population genetics studies.

We report the development of microsatellites for *O. nubilalis* potentially suitable for population studies, and the results of cross-amplification of these markers in the related Asian corn borer, *Ostrinia furnacalis*. We followed the biotin-enrichment methods of Kijas *et al*. (1994) and Ronald *et al*. (2000) with slight modifications as described by Kim & Sappington (2004). Pooled genomic DNA from 30 *O. nubilalis* adults was digested separately with two restriction enzymes, and DNA fragments 250–600 bp long were purified. To increase the chance of finding microsatellites, we constructed two genomic libraries with *Nde*II and *Taq*I. A genomic library was constructed using *Nde*II (or *Sau*3AI) as reported previously (Ronald *et al*. 2000; Kim & Sappington 2004). To construct a double-stranded DNA linker with a *Taq*I compatible end, we used a new primer with 5′-end phosphorylation (5′Phos-CGGATTTTTTATGATGGGCA GGAGGTGGGGG). The *Nde*II and *Taq*I linkers were incubated with the DNA digested with the respective restriction enzymes, and polymerase chain reaction (PCR) amplification was carried out in a Biometra T-gradient thermocycler using the EP-3 primer of Ronald *et al*. (2000). The biotinylated capture probe was annealed to the DNA fragments linkered with an *Nde*II or *Taq*I compatible end. After incubation with magnetic beads, the captured fragments were washed with 1× SSC at an optimized temperature [67 °C $(CA)_{15}$, 62 °C $(CT)_{15}$, 68 °C $(AGC)_7$, 58 °C $(GATA)_6$].

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*Correspondence: Thomas W. Sappington, Fax: 515-294-2265; E-mail: tom.sappington@ars.usda.gov

Table 1 Characteristics of polymorphic microsatellites from European corn borer (*Ostrinia nubilalis*) in a survey of 72 wild individuals from central Iowa, and size range of alleles in cross-amplified loci in Asian corn borer (*Ostrinia furnacalis*) (ACB). H_O, observed heterozygosity. H_E, expected heterozygosity

Locus	Primer sequences (5′–3′)	Repeat motif	No. of alleles	Size range	H_O	H_E	*P**	GenBank Accession no.	ACB†
On-D1	F: GGGCCCAAAAGATAAGGAGA R: CGCACTTAGATTGAGCGAGA	$AA(GA)_3AG$	7	131–153	0.397	0.742	0.0000	EF460498	150–156
On-D2	F: ACTTTTGGCCAAGTGTCCTG R: CGTAGTTGTGACGCTTGGTG	$(TC)_{29}$	22	147–273	0.377	0.934	0.0000	EF636669	174–192
On-D3	F: ATGTGCAACAGGGAGAGCTT R: GCGACTGTAGTAGGGGAGCA	$(TC)_6$	13	161–215	0.423	0.754	0.0000	EF636670	165–171
On-T1	F: ACGAGCTTTATCCGACGTGT R: AGGCCTGAGGAGCAGCAGT	$[G(C/T)T]_{16}ACA[G(C/T)T]_{13}$	3	123–132	0.403	0.327	0.0956	EF460499	123–129
On-T2	F: AAATGGCGTACGAGACGAAC R: ACTGTTGCATGTGAGGGTGA	$(GCA)_2GCC(GCA)_4GCC(GCA)_3GCC[(G/A)CA]_{10}TCAGGC[A(G/A)C]_{10}AGAT(GCA)_3$	2	221–224	0.431	0.422	1	EF460500	219
On-T3	F: GGCAGCTATGGAGGCTAAGA R: CTGGCCCTGCATCTGTTG	$(GCA)_{22}$	6	148–166	0.583	0.621	0.4660	EF460501	151–160
On-T4	F: CTACGAGCCGCACTTGTACC R: CGTGAGAAGCGTCCTACCTG	$(AGC)_2ACC(AGC)_7$	8	165–186	0.667	0.757	0.0430	EF460502	175–184
On-T5	F: CCACAATCCTGCTCTGTAAAA R: AGGAGCAGCAGTTCCTCA	$[G(T/C)T]_{24}$	8	208–229	0.347	0.773	0.0000	EF460503	214–220
On-T6	F: ATCTGTTGCTGCAGGTGCT R: CAGCAGATACCGCACCAG	$[G(C/T)T)]_7$	5	138–153	0.611	0.563	0.2730	EF460504	144
On-T7	F: GTGCATCACCACCTGCTGT R: GCCTACATCAACCAGGCTGT	$(GA)_3(GCT)_6$	6	170–215	0.775	0.659	0.0000	EF636671	167–188

In all cases except for On-T3 and On-T6, the forward primer was the one labelled for PCR.
*Hardy–Weinberg exact test, GENEPOP version 3.1b (Raymond & Rousset 1995).
†PCR was carried out at an annealing temperature of 56 °C in the survey of three ACB individuals.

Three hundred and sixty-eight *Nde*II clones and 158 *Taq*I clones (526 total) were sequenced using a Beckman-Coulter CEQ 8000 Genetic Analysis System. Of these, 305 *Nde*II clones and 131 *Taq*I clones appeared to have simple sequence repeats (SSR). Many sequences overlapped or shared a portion of the same sequences. In some sequences, flanking regions were too close to the SSRs, or the SSRs were very long. Seventy-five clones with unique SSR sequences were chosen to design microsatellite primer sets for PCR amplification. Reaction solutions for PCR consisted of 15 ng genomic DNA, 1.5 mM $MgCl_2$, 200 μM of each dNTP, 0.2 μM of each primer, and 0.5 U *Taq* polymerase in a total volume of 12.5 μL. PCR conditions included denaturation for 3 min at 94 °C, followed by 30 cycles of 1 min at 94 °C, 1 min at 56 °C, and 1 min at 72 °C, and a final extension at 72 °C for 5 min.

Based on the results, the primers of 30 loci (23 *Nde*II clones and 7 *Taq*I clones) were labelled with Beckman-Coulter phosphoramidite flourophores and used to test polymorphism and PCR amplification. Ten of these were polymorphic with discernable bands. Other loci showed monomorphic, multiple or nonspecific bands that made allele calling difficult. One locus (On-D1) was obtained from *Taq*I clones and the other nine loci from *Nde*II clones (Table 1).

Variability of the 10 polymorphic microsatellites was surveyed across 72 wild *O. nubilalis* individuals collected as adults in central Iowa in 2005 (Table 1). The number of alleles per locus ranged from two for On-T2 to 22 for On-D2, and expected heterozygosity values ranged from 0.327 for On-T1 to 0.934 for On-D2. All 10 loci were successfully amplified from *O. furnacalis* (Table 1). Pairwise comparison of locus pairs (45 tests) showed significant evidence of linkage disequilibrium only in one case (On-T6 vs. On-T7) based on Fisher's method using the genotype disequilibrium option implemented in the program GENEPOP (Raymond & Rousset 1995).

Five of 10 loci deviated significantly from Hardy–Weinberg proportions, based on both unadjusted ($P < 0.05$) and adjusted significance thresholds ($P = 0.005$) (Table 1), with a significant deficit of heterozygotes. An exception was On-T7, which showed a significant excess of heterozygotes ($F_{IS} = -0.177$ for On-T7). Analyses of these loci, except for On-T7, using MICRO-CHECKER (Oosterhout *et al*. 2004) also indicated the presence of null alleles. However, the other five loci showed no evidence for null alleles, the preferential amplification of small alleles, or scoring error due to stuttering (Oosterhout *et al*. 2004). Although we caution that the remaining five markers must be examined

carefully in future samples for null alleles and other potential problems commonly observed with microsatellites in Lepidoptera, these additional loci promise to facilitate population genetics studies of European corn borer.

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